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The relationship between annual lung function decline and serum bilirubin levels in chronic obstructive pulmonary disease

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Abstract

Aim: To study the relationship between the level of bilirubin in the serum and the annual decline in lung function in chronic obstructive pulmonary disease (COPD).

Materials and Methods: The medical records of 1,574 patients diagnosed with COPD were reviewed in this retrospective study. In total, 126 eligible patients were included. Data from the initial visits and lung function tests performed at the end of the first year, along with serum direct bilirubin and total bilirubin (TB) measurements, were obtained from the electronic system and analyzed. Pearson correlation was used to determine the association between two continuous parameters.

Results: Negative correlation was found between the annual changes in the first second of forced expiration (FEV_1) percentage, FEV_1 ml, predicted forced vital capacity (FVC) percentage, FVC ml, and predicted forced expiratory flow $(FEF)_{25–75\%}$ percentage and the mean TB values and it was statistically significant (r: -0.202, -0.342, -0.236, -0.287, and -0.136, respectively). However, TB values did not have a significant relationship with the change in the FEV_1/FVC ratio.

Conclusion: The progression speed of COPD may vary among different patients. An elevated serum TB concentration within physiological limits could be considered a parameter that may slow the progression of COPD.

Introduction

COPD is a lung disease characterised by chronic respiratory symptoms such as dyspnoea, cough, production of sputum and/or exacerbations due to disorders in the airways and/or alveoli, typically leading to progressive and persistent airflow obstruction [1]. COPD's high global prevalence, combined with its significant morbidity and mortality rates, imposes a substantial healthcare burden [2].

A critical part in the pathophysiology and progressive nature of COPD is played by chronic inflammation and oxidative stress induced by environmental and endogenous factors [3]. Mediators released by increased macrophages, lymphocytes, and neutrophils in the airways, like tumor necrosis factor-alpha, chemokine ligand 8, chemokine ligand 2, interleukin-1, interleukin-6 and matrix metalloproteinases, are considered responsible for chronic inflammation [4].

Bilirubin is a potent endogenous antioxidant and anti-inflammatory agent, released during the breakdown of the heme molecule and counteracts oxidative damage and helps control oxidative stress by neutralizing free radicals associated with lipid peroxidation [5]. Researchs have shown that elevated levels of bilirubin within physiological limits are correlated with a lower incidence of COPD diagnosis, fewer exacerbations, slower lung function decline, and lower mortality rates [6-8].

The aim of this study was to estimate the association between serum bilirubin levels and annual decline in lung function in COPD.

Materials and Methods

Study subjects

The medical records of patients diagnosed with COPD who applied to the Chest Diseases Clinic of Adana City Training and Research Hospital between January 1, 2022 and January 1, 2023 were reviewed after obtaining ethics committee approval (Adana City Training and Research Hospital Clinical Research Ethics Committee, approval number: 3264). Patient data, including age, gender, smoking history (pack-years), comorbidities, and serum levels of total bilirubin (TB), direct bilirubin (DB), albumin, aspartate

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aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase, were collected from the initial visit and the first-year follow-up. In addition, post-bronchodilator values obtained from pulmonary function tests, namely forced expiratory volume in the FEV_1 (predicted percentage and ml), FVC (predicted percentage and ml), FEV_1/FVC ratio, and $FEF_{25–75\%}$ (predicted percentage), were transferred to a computer environment.

The inclusion criteria were a diagnosis of COPD with a post-bronchodilator FEV1/FVC ratio $<$ 70% on spirometric testing, $\geq$ 18 years old, and a smoking history of $\geq$20 pack-years. The exclusion criteria were the presence of comorbidities that could elevate bilirubin levels, such as hemolytic and hepatobiliary disorders, malignancy or Gilbert's syndrome; TB levels exceeding 1.75 mg/dL in females or 2.34 mg/dL in males; inability to access first-year follow-up data; continued active smoking; failure to receive regular treatment; ongoing exposure to environmental risk factors; and a $<$20 pack-years of smoking in background. Figure 1 presents the patient flowchart.

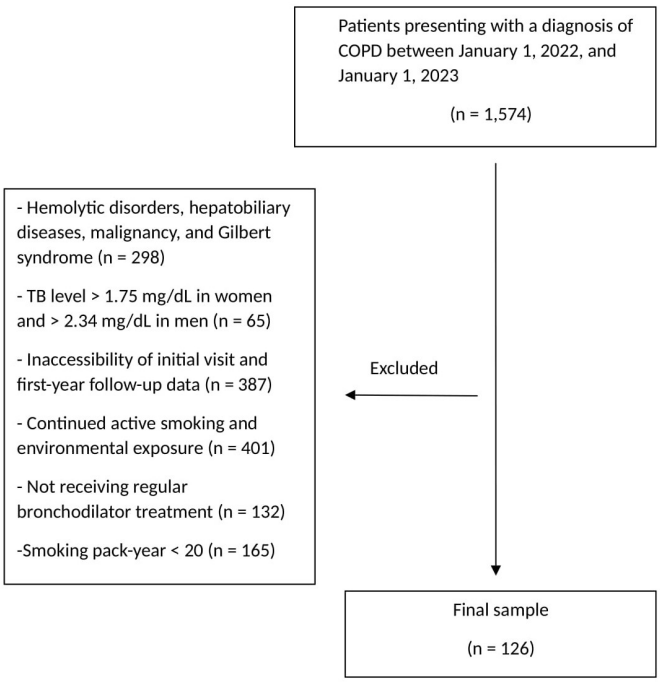

Figure 1. Patient flowchart.

Lung function test

Pre- and post-bronchodilator lung function tests were conducted using the CareFusion spirometer (SentrySuite version 2.19.7, Germany 234 GmbH, Leibnizstraße). Post-bronchodilator measurements were taken 20 minutes after administering 400 µg of salbutamol sulfate aerosol. Testing adhered to maneuver and quality standards set by the European Respiratory Society [9]. Post-bronchodilator values were used in analysis.

The severity of airflow limitation in patients with a FEV_1/FVC ratio $<$ 70% (post-bronchodilator) was classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as follows:

- Stage 1: predicted $FEV_1 \geq 80\%$
- Stage 2: predicted $FEV_1 = 50–80\%$
- Stage 3: predicted $FEV_1 = 30–50\%$
- Stage 4: predicted $FEV_1 \leq 30\%$

Bilirubin measurement

Venous blood samples were collected after an eight-hour fasting period as part of routine practice. Samples were centrifuged at 3,000 rpm at 4 °C, and bilirubin levels were measured using the vanadate oxidase method in the central laboratory.

Statistical analysis

Statistical analyses were conducted using the Statistical Package for the IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was utilized to evaluate the data distribution's normality. Data that followed a normal distribution were expressed as mean ± standard deviation values, Pearson correlation analysis was employed to assess the degree of linear association between two continuous variables. We calculated the sample size using the G-power programme. For the correlation model, the confidence interval was 95% and a standart deviation of 0.5, the effect size was 0.3. Statistical significance was defined as a p-value of less than 0.05.

Results

Of the 126 patients included in the study, 83.3% (n = 105) were male and 16.7% (n = 21) were female, with a mean age of 63.9 years (±8.49, range: 41–85). The mean body mass index was 28.9 (±6.5, range: 21–33), the mean smoking history was 35.5 pack-years (±14.5, range: 20–80), and the mean time since smoking cessation was 6.5 years (±4.5, range: 2–13).

When classified according to the severity of airflow limitation, 25 patients were in stage 1, 32 were in stage 2, 39 were in stage 3, and 30 were in stage 4. The mean baseline spirometric values were as follows: FEV_1/FVC ratio, 57.8% (±11.5); predicted FEV_1 percentage, 54.6% (±18.6); FEV_1 mL, 1,601.4 (±654.9); predicted FVC percentage, 71.3% (±17.4); FVC ml, 2,653 (±941.7); and predicted $FEF_{25–75\%}$ percentage, 53.2% (±15.8).

A significant positive correlation was observed between initial visit TB levels and baseline FEV_1/FVC, predicted FEV_1 percentage, and FEV_1 ml (r = -0.416, -0.346, and -0.284, respectively). However, no significant correlation was found between TB levels and predicted FVC percentage, FVC ml, or predicted $FEF_{25-75\%}$ percentage. A significant positive correlation was also observed between initial visit DB levels and baseline FEV_1/FVC (r = -0.335), although no significant relationship was found with predicted FEV_1 percentage, FEV_1 ml, predicted FVC percentage, FVC ml, or predicted $FEF_{25–75\%}$ percentage (Table 1).

At the first-year follow-up, a significant negative correlation was detected between mean TB levels and the annual changes in predicted FEV_1 percentage, FEV_1 ml, predicted FVC percentage, FVC ml, and predicted $FEF_{25–75\%}$

Table 1. Relationship between baseline pulmonary function test parameters and baseline bilirubin concentrations.

		FEV_1/FVC	Predicted FEV_1 %	FEV_1 ml	Predicted FVC %	FVC ml	Predicted $FEF_{25–75\%}$
Total bilirubin (mg/dl)	r	0.416	0.346	0.284	0.114	0.106	0.214
	p	**0.01**	**0.04**	**0.03**	0.40	0.43	0.09
Direct bilirubin (mg/dl)	r	0.335	0.146	0.200	0.018	0.042	0.132
	p	**0.02**	0.32	0.17	0.90	0.78	0.09

FEV_1: forced expiratory volume in the first second, FVC: forced vital capacity, FEF: forced expiratory flow, r: correlation coefficient. Statistically significant at p<0.05.

Table 2. Relationship between changes in pulmonary function test parameters and bilirubin levels.

		FEV_1/FVC	Predicted FEV_1 %	FEV_1 ml	Predicted FVC %	FVC ml	Predicted $FEF_{25–75\%}$
Total bilirubin (mg/dl)	r	-0.230	-0.202	-0.347	-0.236	-0.287	-0.136
	p	0.88	**0.04**	**0.009**	**0.04**	**0.03**	**0.04**
Direct bilirubin (mg/dl)	r	-0.024	-0.234	-0.076	-0.129	-0.082	-0.213
	p	0.87	0.55	0.08	0.06	0.87	0.09

FEV_1: forced expiratory volume in the first second, FVC: forced vital capacity, FEF: forced expiratory flow, r: correlation coefficient. Statistically significant at p<0.05.

percentage (r = -0.202, -0.342, -0.236, -0.287, and -0.136, respectively), but there was no significant relationship between TB and the change in the FEV_1/FVC ratio. Furthermore, no significant correlation was found between the mean DB levels and the annual changes in FEV_1/FVC, predicted FEV_1 percentage, FEV_1 ml, predicted FVC percentage, FVC ml, or predicted $FEF_{25–75\%}$ percentage (Table 2).

Discussion

This study showed that as mean annual serum TB levels increased, the rate of annual decline in lung function decreased. In COPD, increased inflammation and oxidative stress arise from exogenous sources, such as smoking, or endogenous production. These oxidative products cause damage to DNA, lipids, and proteins [10]. Harijith et al. reported that nicotinamide adenine dinucleotide phosphate oxidase contributed to lung remodeling [11]. Other studies have shown that lipid peroxidation in human lungs leads to cellular membrane component damage, impairing cell structure and permeability [12] and that higher levels of lipid peroxidation products are found in the sputum of patients with COPD [13]. As a result, the remodeling of airways leads to airway obstruction and irreversible limitations detected in pulmonary function tests.

The degradation of heme to biliverdin by hem oxygenase is the first step in the biochemical production of bilirubin [14]. Biliverdin is then converted into bilirubin by biliverdin reductase [15]. Heme oxygenase-1, an inducible isoform, is upregulated by oxidative stress and hypoxia and is produced by type 2 pneumocytes and alveolar macrophages [16, 17]. Increased intracellular biliverdin/bilirubin redox couple protects the vascular endothelium against reactive oxygen and nitrogen [18]. In a COPD rat model study, exogenous bilirubin administration was shown to reduce lung inflammation, suppress regional oxidative lipid damage, and mitigate smoking-induced pulmonary emphysema [19]. A systematic review of 13 observational studies concluded that benign increases in serum bilirubin reduced mortality and had positive effects on lung capacity and COPD prognosis [20].

Apperley et al. and Dai et al. reported a negative correlation between annual FEV_1 decline and total bilirubin concentrations [6, 21]. Consistent with the literature, our study revealed that as the annual mean TB levels in patients with COPD decreased, there was an increase in FEV_1 loss (in both predicted percentage and milliliters) during pulmonary function tests. This finding suggests that physiological levels of serum TB may slow remodeling through its antioxidant effects and help prevent functional decline.

In contrast to expectations, another study found no significant relationship between TB and the FEV_1/FVC ratio in patients with COPD [21]. In our study, while a positive correlation was detected between baseline FEV_1/FVC values and TB, there was no relationship between mean TB and annual FEV_1/FVC changes, which is consistent with the literature.

Our results also demonstrated a relationship between baseline FEV_1 values and mean serum TB levels. Supporting our findings, Leem et al. found that higher bilirubin concentrations were associated with higher FEV_1, longer six-minute walking test distances, and better quality of life [22]. These results suggest that an increase in TB concentrations may limit remodeling from the early stages through its anti-inflammatory and antioxidant effects, thus preventing loss of lung capacity.

In this study, while no significant relationship was found between TB and baseline $FEF_{25–75\%}$, there was a correlation between the annual change in $FEF_{25–75\%}$ and mean serum TB levels. Similarly, Curjuric et al. reported a significant relationship between serum bilirubin levels and FEV_1/FVC and $FEF_{25–75\%}$ in non-smokers [23]. $FEF_{25–75\%}$ is used in pulmonary function tests as a parameter representing small airway diseases. Due to the anti-inflammatory effects of TB, particularly at the alveolar and bronchiolar levels, it may limit the decline in

$FEF_{25–75\%}$.

Limitations

First, we did not have data on patients' pulmonary function tests or lung capacity before COPD, making it difficult to comment on the impact of TB on the onset of the disease. Second, we did not exclude genetic factors that may facilitate the progression of the disease and loss of lung capacity in patients with COPD. To address these limitations, there is a need for long-term prospective studies with larger patient groups that exclude modifying factors.

Conclusion

COPD is an airway disease characterized by irreversible airway obstruction and progressive loss of lung capacity. Patients' illness progressions differ from one another. Higher serum TB concentrations within physiological limits can be considered one of the parameters that slows the progression of COPD.

Ethics Committee Approval

The research was approved by the Clinical Research Ethics Committee of Adana City Training and Research Hospital on 28 March 2024 with the approvel number 3264. Throughout the trial, researchers ensured the confidentiality of patient data and adhered to the ethical principles of clinical research as outlined in the Declaration of Helsinki. Due to the retrospective nature of the study, the ethical committee waived the need for informed consent.

Consent for Publication

All co-authors have seen and approved the contents of the manuscript. We confirm that this manuscript is our own original work and is not currently under consideration by any other publication. Data available on consent due to privacy/ethical restrictions.

Competing Interests

The authors certify that they have no conflicting interests with regard to this study or its publication.

Data Availability

We agree to keep records for at least 5 years. All data generated in the study will be made available to the corresponding author upon reasonable request.

Author Contributions

Concept: SBS, OED, acquisition of subjects and/or data: OED,MA, analysis and interpretation of data: SBS, SK, MA and preparation of study: SBS, SK, MA

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